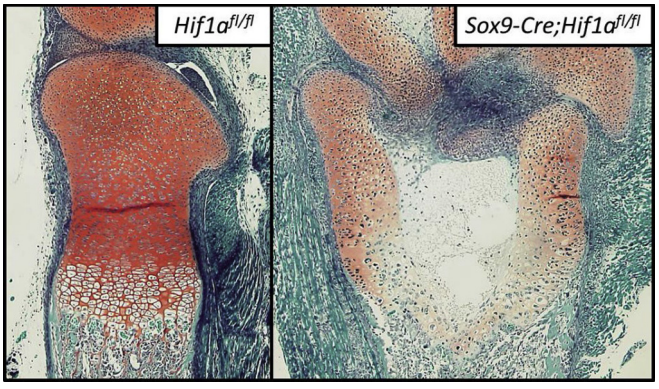
showed a decrease in proteoglycan content in comparison with the control Hif1afl/fl embryo. TUNEL staining accentuated ectopic apoptosis broadly across the limb cartilage, and immunofluorescence showed enhanced expression of Mmp13 surrounding the defective area. When we created the experimental OA model one week after the tamoxifen injection into Col2a1-CreERT2;Hif1afl/fl mice, the OA development in the knee joints was markedly accelerated as compared to the control Hif1afl/fl joints. Apoptosis and Mmp13 expression were upregulated by the conditional knockout of HIF-1α after maturation in articular cartilage, as well as in the limb cartilage. To reveal altered gene expression by HIF-1α deficiency, we obtained RNA samples directly from the limb cartilages of Sox9-Cre;Hif1afl/fl and the control Hif1afl/f embryo. Real-time RT-PCR using these samples revealed increases of catabolic factors including Mmp13 and Mmp9, and decreases of anabolic factors including Col2a1 and Sox9 by the conditional knockout of HIF-1α. When we deleted HIF-1α in primary articular chondrocytes from Hif1afl/fl mice by adenoviral vector expressing Cre recombinase, expressions of the catabolic and the anabolic genes were changed in ways similar to those in the in vivo analyses, under both normoxic and hypoxic (1% O2 concentration) conditions. Similar results were also obtained under both O2 conditions by HIF-1α silencing in primary articular chondrocytes from WT mice using siRNA. Furthermore, in the organ culture of mouse femoral heads, stabilizing HIF-1α protein by CoCl2 treatment markedly decreased aggrecan release into the medium.

Conclusions: HIF-1α regulates the configuration and maintenance of articular cartilage through induction of anabolic factors and suppression of catabolic factors. Elucidation of the molecular network related to HIF-1α may lead to cartilage regeneration and OA treatment.



273
DNA REPAIR ENZYME, APURINIC/APYRIMIDINIC ENDONUCLEASE 2 (APEX2), HAS A POTENTIAL TO PROTECT AGAINST THE DOWN-REGULATION OF CONDROCYTE ACTIVITY IN OSTEOARTHRITIS

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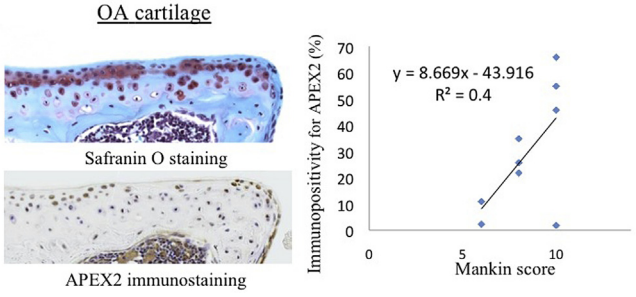
Background: Recent reports clearly indicate that chronic excess production of reactive oxygen species (ROS) from chondrocytes, which is induced by mechanical force to cartilage, plays an important role in cartilage degeneration occurring after mechanical injury to cartilage in osteoarthritis (OA). However, pathogenic mechanism of ROS-mediated degeneration of articular cartilage remains unknown. While studies have provided ample confirmation of the generation of ROS in OA cartilage, the activity of cellular antioxidants in degenerated articular cartilage still remains unclear.

Propose: Apurinic/apyrimidinic endonuclease 2 (Apex2) is an essential DNA repair enzyme that plays a critical role in DNA repair against the oxidative damage in a variety of human somatic cells. We postulated that Apex2 in chondrocytes may have a role to protect against the catabolic process of articular cartilage in OA. The aim of the study was to examine the potential involvement of DNA repair enzyme Apex2 in the pathogenesis of OA.

Methods: Expression of Apex2 was histologically investigated in OA articular cartilages from STR/OrtCrlj mice, an experimental animal model which spontaneously develops an osteoarthritic process. We examined whether OA-related catabolic factor [interleukin (IL)-1β] influenced the expressions of Apex2 in human chondrocytes. Knockdown of Apex2 with small interfering RNA (siRNA) was also performed to investigate whether Apex2 is associated with cellular activity and survival in human chondrocytes.

Results: In OA mouse chondrocytes, higher levels of Apex2 expressions were histologically observed in the severe OA cartilages than in mild degenerated cartilages. The immunopositivity of Apex2 was significantly correlated with the degree of cartilage degeneration (Figure). OA-related catabolic factor, IL-1β, induced the expression of Apex2 in chondrocytes. Apex2 silencing using siRNA reduced the chondrocyte activity in vitro.

Conclusions: The expression of DNA repair enzyme Apex2 in chondrocytes was associated with the degeneration of articular cartilages and was induced by OA-relating catabolic factor. Our findings suggest that Apex2 may have a potential to prevent the catabolic stress-mediated down-regulation of chondrocyte activity in OA.



274
HYPERGLYCEMIA-LIKE CULTURE CONDITIONS INDUCE IL-1B AND TNF-α EXPRESSION AND IMPAIR AUTOPHAGY IN HUMAN CHONDROCYTES

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Purpose: Accumulating evidence indicates that Diabetes Mellitus (DM) is an independent risk factor for severe osteoarthritis (OA). Understanding the mechanisms involved is essential for designing preventive strategies and targeted therapies that can halt OA progression in DM patients. In this context, we hypothesized that hyperglycemia, a hallmark of DM and other conditions associated with glucose imbalance, is a major effector of chondrocyte damage. Accordingly, our previous studies showed that culture of human chondrocytes under excess glucose favors catabolic responses and oxidative stress. This study aims at further characterizing the effects of hyperglycemia in human chondrocytes by determining whether it also promotes inflammatory responses. Furthermore and to gain some insight as to the mechanisms by which hyperglycemia favors OA progression, modulation of autophagy was also evaluated as a crucial mechanism for the elimination of damaged proteins and organelles whose impairment has been implicated in the deleterious effects of hyperglycemia in various cells.

Methods: Articular cartilage was obtained from multi-organ donors (44-73 years old, mean = 59.4, n = 10) at the Bone Bank of the University and Hospital Center of Coimbra with approval by the Ethics Committee. Isolated chondrocytes and the human chondrocytic cell line, C28/I2 (kindly provided by Prof. Mary Goldring and Harvard University), were cultured in Ham-F12 or DMEM:Ham F-12 (1:1), respectively, containing regular (10 mM) or excess (30 mM) glucose for various periods. The expression of pro-inflammatory markers (IL-1β and TNF-α) was evaluated by qRT-PCR. Autophagy was assessed by determining the protein levels of LC3-I and II in the presence and absence of the lysosome inhibitor, chloroquine. To rule out possible osmotic effects, parallel experiments were performed in the presence of the cell-impermeable polyol, mannitol. Cell viability was evaluated by the MTT reduction assay.

* These authors contributed equally to this work